ORAL COMMUNICATIONS / INCORPORATIONS

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1.
REGULACIÓN DE FACTORES DE TRANSCRIPCIÓN POR MIRNAS SENSIBLES A ESTRADIOL EN CÉLULAS ENDOTELIALES HUMANAS Regulation of transcription factors by estradiol-sensitive miRNAs In human endothelial cells.

Estrogens, mainly 17beta-estradiol, confer a protective role in cardiovascular biology primarily by regulating endothelial function, with several studies highlighting the role of transcription factors in this protective effect. In addition to transcription factors, microRNAs (miRNAs), small non-coding RNAs, emerge as pivotal regulators of gene expression and signaling networks, exhibiting the ability to influence the activity of hundreds of genes. miRNAs bind to complementary sequences on mRNAs, typically leading to mRNA degradation or translation repression. To identify regulatory networks of miRNA and transcription factors regulated by estradiol in human endothelial cells, we performed an integrative bioinformatic analysis in which we constructed miRNA-mRNA interactions (Ingenuity Pathway Analysis software) using our prior mRNA and miRNA microarray data from human umbilical vein endothelial cells (HUVEC) treated with 1 nM 17beta-estradiol for 24 h (Sobrino, A. et al. 2009, PloS One, 4: e8242; Vidal-Gómez et al. 2018; Cell. Physiol. Biochem. 45(5): 1878, respectively). We also identified transcription factors regulated by estradiol-sensitive miRNAs. The analysis identified 102 dysregulated estradiol-sensitive miRNAs that were paired with 588 inversely expressed estradiol-sensitive mRNAs. The functional characterization of the miRNA/mRNA association analysis highlighted canonical pathways regulated by 17beta-estradiol in HUVEC, including hypoxia signaling in the cardiovascular system, ILK signaling, integrin signaling, ephrin receptor signaling, and regulation of actin-based motility by Rho. The transcription factors and downstream genes analysis revealed eight networks, including JUN which was regulated by miRNA-30b-5p and REPIN1, regulated by miR-25-5p, both associated with typical endothelial functions such as cadherin binding and cell adhesion molecule binding pathways. In conclusion, through an integrative microarray analysis, we have identified regulatory networks through which estradiol could regulate vascular function in human endothelial cells.

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2.
ROL DE ADENOSINA TRIFOSFATO (ATP) Y ÁCIDO LINOLEICO EN LA RESPUESTA INFLAMATORIA DE CÉLULAS ENDOMETRIALES BOVINAS. Role of adenosine triphosphate (ATP) and linoleic acid on inflammatory response in bovine endometrial cells.

Endometrial cells play a key role in the inflammatory response, in addition to their physiological reproductive function. Endometrial cells are exposed to molecules such as adenosine triphosphate (ATP), and especially in cows, to linoleic acid. Linoleic acid, a long chain fatty acid, increases in blood in cows postpartum, and ATP is a well-known initiator and regulator of the inflammatory response. However, the role of linoleic acid and ATP in inflammatory response in endometrial cells is not clear. The aim of this study was to determine the inflammatory response-associated effects of linoleic acid and ATP on bovine endometrial cells. Bovine endometrial (BEND) cells were stimulated with linoleic acid and extracellular ATP was measured with a luminescent assay. The effect of ATP and linoleic acid on interleukin-8 (IL-8) and IL-6 were determined by ELISA assay. In addition, the effect of ATP and linoleic acid on intracellular calcium was assessed by spectrofluorimetry. Finally, we assessed a possible effect of linoleic acid on changes in metabolic routes, through metabolomic assay. We observed that linoleic acid significantly increased ATP levels at 15 s of stimulation. On the contrary, linoleic acid did not increase IL-8 and IL-6 in BEND cells, however when linoleic acid was incubated in presence of Lipopolysaccharide (LPS), a reduction in IL-8 and IL-6 levels was observed. The metabolomic assay showed that linoleic acid and linoleic acid plus LPS induced metabolomics changes compared with the control. ATP induced high levels of IL-8 and IL-6, and increased intracellular calcium. In conclusion, ATP and linoleic acid induce pro-inflammatory responses in bovine endometrial cells, however linoleic acid also could have an anti-inflammatory effect in cells treated with LPS. Therefore, the mechanism by which linoleic acid induce its effects remain to be studied.

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3.

INHIBICIÓN FARMACOLÓGICA DEL COMPLEJO HSC70/LAMP2A PARA EL DESARROLLO DE POTENCIALES DROGAS ANTICANCERÍGENAS. Pharmacological Inhibition of the HSC70/LAMP2A Complex for the Development of Potential Anticancer Drugs.

Chaperone-Mediated Autophagy (CMA) is a process in which proteins bearing the KFERQ motif are translocated through the lysosomal membrane for subsequent degradation. The key proteins responsible for this process are HSC70, a chaperone responsible for motif recognition, and LAMP2A, a transmembrane protein that interacts with HSC70 and forms the pore to allow the internalization of the target protein. CMA has been reported to be highly activated in various types of cancer, whether in tissues or cell cultures, which provides protection against hypoxia and nutrient deprivation among other advantages. Inhibition of this pathway has shown to block the growth and metastatic properties of tumors in animal models. In this work, we propose the development of drugs with the ability to inhibit the HSC70/LAMP2A complex formation as a potential cancer treatment. In this study, comparative modeling and molecular dynamics were used to obtain the most probable conformations of human HSC70 and LAMP2A. Subsequently, a model of the complex was constructed through docking, inserted into a lysosomal membrane, and subjected to molecular dynamics to assess its stability. Finally, once a stable complex was obtained, docking was performed on the interaction portion of HSC70 using the MCULE and NCI databases, which collectively comprise over 3 million compounds. The results obtained were then filtered, considering ADME properties, yielding several molecules with a high likelihood of inhibition, of interest for an analog program, and thus a new therapeutic option.

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4.

SENESCENCIA INDUCIDA POR TGF-B1 EN FIBROBLASTOS CARDÍACOS. TGF-β1-induced senescence in cardiac fibroblasts.

Cardiac fibroblast (CF) to cardiac myofibroblast (CMF) differentiation is triggered by TGF-β1, and it is a critical process for cardiac tissue repair after damage. TGF-β1 has been reported to induce cell cycle arrest in cancer cell lines, which is an essential feature of cellular senescence. Senescent cells are characterized by the expression of cyclin-dependent kinase inhibitors, an increase in lysosomal content, which is key to assessing senescence-associated β-galactosidase activity (SA-β-Gal), the acquisition of a senescence-associated secretory phenotype (SASP) and being resistant to apoptosis through the expression of antiapoptotic proteins. Aims: Determine whether TGF-β1 induces CF senescence, evaluate which senescence pathways might be involved in this response, and determine if senescence induction alters CMF functionality. Methodology: Primary cultures of neonatal rat CF were maintained in medium supplemented with 10% serum and stimulated with TGF-β1 for 7 days. After the experimental times, the expression levels of p21, p15-p16, p-Rb, collagen I, Bax, and Bcl-xL proteins were evaluated by western blot. Additionally, α-SMA protein assembly was evaluated by immunofluorescence. Finally, SA-β-Gal activity was evaluated by cytochemistry. Results: TGF-β1 treatment increased p21, p15-p16, and collagen I levels, whereas a decrease in Rb phosphorylation was observed, which is consistent with senescent phenotypes. No changes were observed in Bax and Bcl-xL expression levels. At the same time, immunofluorescence showed the assembly of α-SMA in stress fibers, which is a marker of CF-to-CMF differentiation. In SA-β-Gal staining, stimulation with TGF-β1 increased the number of staining-positive cells at the end point. Conclusion: Our results suggest that TGF-β1 induces CMF differentiation as well as CMF senescence. Functionally, these senescent cells produce a higher content of collagen I.

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